NANOPROBES FOR DETECTION OR MODIFICATION OF MOLECULES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Nos. 60/749,729 and 60/749,858 both filed Dec. 12, 2005 and herein incorporated by reference.

FIELD

[0002] This application relates to probes that can be used to detect or modify molecules, such as proteins and nucleic acid molecules, as well as methods of their use.

BACKGROUND

[0003] Several methods are currently available for detecting proteins and other molecules (such as nucleic acid molecules). For example, proteins can be detected using western blotting, flow cytometry, and ELISA methods. In addition, nucleic acids can be detected using Southern or northern blotting, microarrays, quantitative or non-quantitative PCR, chemical footprinting, and other methods known in the art. However, these methods require multiple steps and long detection times. Therefore, agents that permit detection with fewer steps and less time are needed. In addition, agents that permit detection in vivo are needed.

[0004] Methods are also currently available for modifying proteins and nucleic acid molecules, such as the use of antisense or siRNA molecules. However, agents having broader applications are needed.

SUMMARY

[0005] The disclosure is directed to molecular agents (referred to herein as nanoprobes) that can be used for detecting (such as quantitating) or modifying (such as destroying) one or more target molecules, such as biomolecules, organic molecules, and other molecules such as nylon. For example, the probes can be used to detect or modify a protein or a nucleic acid molecule. Although the application provides probes to use for particular biomolecules, one skilled in the art will recognize that the disclosed probes can be adapted to detect or modify any molecule of interest, for example by using particular functional groups.

[0006] In one example, a probe for a target biomolecule includes a molecular linker and first and second functional groups linked and spaced by the molecular linker. The functional groups are capable of interacting with one another or with the target biomolecule in a predetermined reaction, wherein the molecular linker links the first and second functional groups sufficiently spaced from one another such that the functional groups do not substantially interact in an absence of the target biomolecule. In particular examples, the molecular linker links the first and second functional groups sufficiently spaced a distance from one another to avoid substantial entanglement of the first and second functional groups in an absence of the target biomolecule. In some examples, the molecular linker (or at least a portion thereof) is of sufficient rigidity to reduce interaction of the first and second functional groups in the absence of the target biomolecule. In particular examples, the molecular linker is of a sufficient length to substantially avoid interaction of the first and second functional groups in the absence of the target biomolecule, and allow interaction of the first and second functional groups in the presence of the target biomolecule.

[0007] The molecular linker (or a portion thereof, such as a molecular rod that is part of the molecular linker) has a sufficient length in view of its flexibility to space the functional groups sufficiently apart to avoid the undesired interaction in the absence of the target biomolecule, but retain sufficient flexibility to allow the functional groups to interact with each other or the target when one or more functional groups bind to the target. For example, at least part of the linker can have a persistence length that permits at least a portion of the molecular linker to be of sufficient rigidity and length to reduce interaction of the first and second functional groups in the absence of the target biomolecule, and allow interaction of the first and second functional groups in the presence of the target biomolecule. In particular examples, the total length of the molecular linker is different than (such as greater or less than) the persistence length of one or more components that make up the linker, such as a double- or single-stranded nucleic acid molecule. However, in particular examples, the total length of the molecular linker does not exceed a length beyond which significant interaction occurs between the first and second functional groups in the absence of the target biomolecule, while allowing significant interaction of the first and second functional groups in the presence of the target biomolecule. Such interactions can be measured using methods known in the art, for example by measuring acceptor emission fluorescence when one functional group includes a donor fluorophore and one or more other functional groups include a corresponding acceptor fluorophore of a FRET pair. In other examples, a functional group is substantially maintained at a distance of at least twice the Förster radius (such as a Förster radius of 22 to 90 Å) from the other functional group in the absence of the target.

[0008] Persistence length (lp) is the average local conformation for a linear chain, which reflects the sum of the average projections of all chain segments on a direction described by a given segment. Therefore, persistence length is a measure of the rigidity or stiffness of a polymer chain. In particular examples, persistence length is the degree of bending (and hence the effective stiffness of the chain) which, in effect, measures the contour distance over which there occurs, on the average, a 68.40° bend. Therefore, the persistence length will vary depending on the composition of the molecular linker. For example, the persistence length for a double-stranded DNA (dsDNA) molecule will differ from that of a single-stranded DNA (ssDNA) molecule and from polyethylene glycol (PEG). In particular examples, dsDNA has a persistence length of about 400-500 Å, and dsRNA has a persistence length of 700-750 Å, for example at an ionic strength of about 0.2 M and at a temperature of 20° C. In particular examples, ssDNA has a persistence length of about 40 Å (for example at 20° C.) (Clossey and Carlon, *Phys. Rev. E. Stat. Nonlin. Soft. Matter. Phys.* 68(6 Pt 1):061911, 2003). In particular examples, PEG has a persistence length of about 3.8 Å.

[0009] Particular examples of molecular linkers include, but are not limited to, tethers, molecular rods, or combinations thereof. For example, the molecular linker of sufficient rigidity can include a molecular rod, for example a molecular rod composed of a double-stranded DNA molecule (dsDNA). In some examples, the molecular linker of sufficient rigidity includes multiple molecular rods linked by tethers, or multiple tethers linked by molecular rods. One particular example